STATUS OF AMENDMENTS

There is presently one amendment pending in this application. The amendment is responsive to the Final Action dated February 3, 2000, amending Claims 1, 3-5 and 26. New Claims 37-42 are submitted and Claim 30 is canceled. Support for the amendment may be found in the specification and in the claims of the application as filed.

SUMMARY OF THE INVENTION

The present invention provides reagents and methods which comprise a system for the rapid subcloning of nucleic acid sequences *in vivo and in vitro* without the need to use restriction enzymes. The present invention provides a method for the recombination of nucleic acid constructs, comprising: providing a first nucleic acid construct comprising, in operable order, an origin of replication, a first sequence-specific recombinase target site, and a nucleic acid of interest, a second nucleic acid construct comprising, in operable order, an origin of replication, a regulatory element and a second sequence-specific recombinase target site adjacent to and downstream from the regulatory element, and a site-specific recombinase; contacting the first and the second nucleic acid constructs with the site-specific recombinase under conditions such that the first and second nucleic acid constructs are recombined to form a third nucleic acid construct, wherein the nucleic acid of interest is operably linked to the regulatory element. The present invention contemplates the use of any type of regulatory element. In some embodiments of the present invention, the regulatory element comprises a promoter element, a fusion peptide (e.g., an affinity domain), or an epitope tag. In preferred embodiments, the nucleic acid of interest comprises a gene.

In some embodiments, the first nucleic acid construct further comprises a selectable marker. In other embodiments, the second nucleic acid construct further comprises a selectable marker. The present invention contemplates that the first and second nucleic acid constructs both comprise selectable markers. In preferred embodiments the selectable markers of the first and second nucleic acid constructs are different from one another. Selectable markers include, but are not limited to a kanamycin resistance gene, an ampicillin resistance gene, a tetracycline resistance gene, a chloramphenicol resistance gene, a streptomycin resistance gene, a spectinomycin resistance gene, the *aada* gene, the (DX174 E gene, the *stra* gene, and *the sacb gene.*

The present invention also provides a nucleic acid construct comprising, in operable order: a conditional origin of replication; a sequence-specific recombinase target site having a 5' and a 3' end; and a unique restriction enzyme site, said restriction enzyme site located adjacent to the 3' end of the sequence-specific recombinase target site. In some embodiments, the construct further comprises a prokaryotic termination sequence. In yet other embodiments, the construct further comprises a eukaryotic polyadenylation sequence. The present invention contemplates the use of any prokaryotic termination sequence and any eukaryotic polyadenylation sequence. In preferred embodiments, the construct further comprises one or more selectable marker genes. Selectable marker genes include, but are not limited to the kanamycin resistance gene, the ampicillin resistance gene, the tetracycline resistance gene, the chloramphenicol resistance gene, the streptomycin resistance gene, the *stra* gene, and the *sacb* gene. In preferred embodiments, the sequence-specific recombinase target site is selected from the group consisting of *loxp, loxP2, loxP3, loxP23, loxP51 1, loxb, loxC2, loxl, loxr, loxA86, loxa 1* 17, *ftt, dif, loxh and att.*

In some embodiments the construct further comprises a gene of interest inserted into the unique restriction enzyme site. In particular embodiments, the construct has the nucleotide sequence set forth in SEQ ID NO:1 (Figure 26A). In other embodiments, the construct further comprises a second sequence-specific recombinase target site. In preferred embodiments, the second sequence-specific recombinase target site is selected from the group consisting of RS site and a Res site. In yet other embodiments, the construct further comprises a polylinker.

The present invention further provides a nucleic acid construct comprising in 5' to 3' operable order: an origin of replication; a promoter element having a 5' and a 3 end; and a sequence-specific recombinase target site having a 5' and a 3' end. In some embodiments, the construct further comprises a selectable marker gene.

The present invention also provides a nucleic acid construct comprising in operable order: a promoter element having a 5' and a 3' end; a first sequence-specific recombinase target site having a 5' and a 3' end, wherein the 3' end of the promoter element is located upstream of the 5' end of the sequence-specific recombinase target site; a gene of interest joined to the 3' end of the sequence-specific recombinase target site such that a functional translational reading frame is created; a conditional origin of replication; a first selectable marker gene; a second sequence-specific recombinase target site; and an origin of replication. In some embodiments, the construct further comprises a second selectable marker gene.

REMARKS

Applicants respectfully request that the present amendment after final be approved by the Examiner and entered. Applicants respectfully submit that the present amendment is in compliance